VALIDITY AND RELIABILITY OF THE MAX II METABOLIC CART FOR MEASUREMENT OF RESTING METABOLIC RATE

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ABSTRACT

Objective: The objective of this study was to assess the accuracy and precision of the Max II Metabolic Cart against the Deltatrac II^{TM} for measurement of resting metabolic rate (RMR).

Materials/Methods: Twenty-two subjects completed 6 resting metabolic rate tests (2 on a Deltatrac II cart and 4 on two Max II carts) over 2 testing days performed approximately 1 week apart in this randomized, crossover study. Each test was performed under fasting conditions.

Results: The 3 carts tests produced reliable measurements of VO_2 and VCO_2 (within subject CV <5%). In comparison to the reference cart Deltatrac II, Max II – Cart A obtained accurate and precise measurements in individual subjects and for the combustion of ethanol. Max II – Cart B, performed with significantly lower levels of accuracy and precision compared to both the reference standard and Max II – Cart A.

Conclusions: Measurements of VO_2, VCO_2, RQ and RMR derived from the Max II metabolic cart are valid in adults compared to our best performing cart, Deltatrac II. Laboratories should adopt standard operating procedures for assessments and quality control testing that can assess the precision of the O_2 and CO_2 analyzers such as ethanol combustion tests.

Key Terms: indirect calorimetry, resting metabolic rate, Deltatrac II^{TM}, Max II

List of abbreviations: VO_2: Volume of oxygen, VCO_2: volume of carbon dioxide, RQ: respiratory quotient, RMR: resting metabolic rate, DXA: dual X-ray absorptiometry.

Measurement of energy expenditure in humans was pioneered by Wilbur Olin Atwater, Edward Bennett Rosa and Francis Gano Benedict at the turn of the 20th century with the development of the first respiration calorimeters [1-3]. At that time, the technique was mostly used to describe the resting metabolic rate and the thermic effect of foods to better understand energy expenditure (EE) in humans; specifically, how it differed between sexes and changed across the lifespan. In response to the rise in obesity, there is now an increasing interest to understand the role of EE to energy balance and interventions to net substrate oxidations to test if newly developed pharmaceutical agents developed for obesity management can stimulate thermogenesis. Indirect calorimetry thereby serves an important role now both clinically and scientifically.

Resting metabolic rate (RMR) is the energy expended to maintain the body's organs and tissues functioning at rest, awake and in a comfortable environment. RMR accounts for approximately 60-70% of total daily energy expenditure in sedentary adults [4]. RMR is related to the body size, and its variability can be explained by individual differences in body composition (fat-free mass and fat mass), gender and age [5-9]. Technological advances late in the last century allowed RMR to be assessed through portable calorimeters at the patient's bedside. Given that RMR testing is noninvasive, and generally a short procedure that can be performed with minimal risk and burden to the subjects, there is now widespread use of the technique in both clinical and scientific settings.

Datex-Ohmeda (Helsinki, Finland) developed the Deltatrac, a portable metabolic cart in the 1980's. The release of their follow-up instrument in the early 90's, the Deltatrac II, provided 3 different flow rate settings to allow for RMR assessments in pediatric, adult and obese populations. For the past 3 decades, the Deltatrac has acquired the reputation of being the most simple, valid and reliable portable calorimeter available and is often benchmarked as the gold standard instrument for RMR assessment [10]. Some institutions have been able to maintain operable Deltatrac's, but without technical support to replace aging parts their existence is becoming obsolete thus creating a void for Investigators and Clinicians seeking a validated and reliable calorimeter [10]. Various companies have attempted to bring new instruments to market although none have approached the good performance of the Deltatrac II™. After road testing several of these new devices, Pennington Biomedical Research Center (Baton Rouge, Louisiana) purchased the new Max II Metabolic Cart (AEI Technologies, Naperville, IL) with the aim to eventually replace all the Deltatrac II™ carts currently in service at the Center.

The aim of this study was to evaluate the accuracy and precision of the Max II metabolic cart against the long-standing gold standard portable indirect calorimeter, the Deltatrac II. Instrument performance was also evaluated using ethanol combustion tests.

METHODS

Subjects. Between March 2011 and May 2012, 22 individuals were enrolled in a study to assess the validity and reliability of the Max II Metabolic Cart at Pennington Biomedical. Subjects were recruited through targeted email advertising calling for a diverse study population; healthy males and females aged 18 to 75 with a wide body mass index range. Subjects were excluded for recent changes in body weight (>5lb fluctuation within 30 days prior to enrollment), claustrophobia, irregular menstrual cycles, smoking, pregnancy or lactation. This study was approved and monitored by the Institutional Review Board at Pennington Biomedical and all subjects provided written informed consent prior to study initiation.

Study Design. In a randomized, crossover study design, subjects completed 6 RMR tests over 2 testing days performed approximately 1 week apart (Figure 1). Each testing day, RMR was measured on two Max II metabolic carts (Max A and Max B) as well as the Deltatrac II metabolic cart. The order of the metabolic cart assignment was determined at random and counterbalanced. We assessed measurement of oxygen consumption (VO_2), carbon dioxide production (VCO_2), respiratory quotient (RQ) and RMR. On each testing day, subjects were asked to arrive to the metabolic ward fasted (at least 10 hours) and after having refrained from alcohol, exercise, and caffeine for the 24 hours prior. Female subjects were tested during the follicular phase of the menstrual cycle or during the hormonal phase of hormonal contraceptive.

Body weight and composition assessments. Upon arriving to the clinic, subjects were asked to void and change into a hospital gown. Body weight was then measured and the gown weight subtracted for a metabolic weight. At visit 1, percent body fat was measured by dual X-ray absorptiometry using the iDXA whole-body scanner (General Electric, Milwaukee, WI). Fat mass and fat-free mass were calculated from total fat percentage and the measured metabolic weight.

Resting Metabolic Rate Assessment. On each test day, subjects completed 3 consecutive RMR tests each using a different metabolic cart. The assessment was conducted in a private, quite room with controlled ambient conditions. Each RMR test was performed for 30 minutes under standardized conditions. Site specific standard operating procedures as well as best practice methods from Comper, et al were followed [11]. First, subjects rested for 30 minutes undisturbed while supine and reclining in a hospital bed at a 30^{o} incline. At the completion of the resting period, the canopy was placed over the subject's head and secured with plastic around the subject's pillow. Subjects were instructed to remain still, awake and to refrain from fidgeting as much as possible. No talking, reading, TV viewing was permitted for the duration of the test. Trained research specialists observed the tests and recorded events such as a cough, sneeze, or yawn that may potentially affect the RMR measurement. In between each test, subjects were allowed to talk but remained on the bed while the next cart was calibrated and prepared for testing.

Prior to each test, each cart was allowed to warm-up according to the manufacturers' instructions (Deltatrac for 30 minutes and Max II for 4 hours). Gas analyzers were then calibrated with room air and gases of known concentrations. For the Deltatrac II, the flow meter was set to the adult setting (40L/min) for all tests and was calibrated prior to each testing by imputing current ambient conditions (temperature, relative humidity, barometric pressure) and calibration with room air and a medical grade calibration gas comprised of 5% CO_2 and 95% O_2 (Gulf Gases, Gonzales, LA). The Max II instrument was calibrated with current ambient conditions (temperature, humidity, pressure), with room air and two certified gases comprised of 1% CO_2 and 21% O_2 for Gas 1 and 0.03% CO_2 and 19% O_2, for Gas 2 (Airgas Specialty Gases, Inc., Lenexa, KS). The flow meter was calibrated with a 3-L Hans Rudolph calibration syringe. During testing, the flow rate was adjusted as necessary throughout each measurement to ensure that $FeCO_2$ was maintained between 0.6 - 0.95%.

Quality Control Testing. Quality control tests were performed on each cart following the standard calibration procedure by measuring the combustion of 5mL of pure ethanol: 100% ethanol was burned to completion at a constant flow rate of 22-25 mL/min. Measured VO_2, VCO_2 and RQ are then compared to theoretical values assuming complete combustion of ethanol (3820 ml of O_2 and 5730 ml of CO_2 per 5 ml ethanol). The expected results from the complete combustion of 5mL of ethanol are 100±5% recovery for oxygen and carbon dioxide and a calculated respiratory quotient of 0.67±0.03.

Statistical Analysis. To assess accuracy and precision of the Max II cart, statistical analyses were performed on VO_2 and VCO_2 data, as well as some standard calculations in which these data are used, RQ and RMR. To test accuracy and precision, each of the variables from the Max II carts were plotted versus Deltatrac II and a linear regression was performed. The intercept and slope of the regression were tested versus one (i.e. line of identity) and zero, respectively. Then, mixed-effect models and multiple comparisons with user-defined contrast matrix and Tukey correction of p-values were used to compare each of the Max II to the Deltatrac II. To assess reliability, intra-class correlation coefficients and within-subject coefficient of variation were calculated within cart over the 2 visits and compared to the Deltatrac II using paired t-tests. The Bland-Altman technique was used to examine any bias between the Deltatrac II and each Max II [12]. Statistical analyses were performed using “R” version 2.14.1 [13]. Statistical significance was considered at $p<0.05$. Data are presented as means ± SD.

RESULTS

Subject Characteristics. A diverse population was successfully recruited with population age ranging from 20 to 59, BMI ranging from 19.2 to 42 kg/m^2, and African American, Asian and Caucasian ethnicities represented. Subject characteristics are summarized in Table 1.

Precision (Quality Control Testing). The precision of the oxygen and carbon dioxide analyzers was assessed with a standardized ethanol combustion test. Reproducibility was evaluated using 10 tests on each cart over 60 days and repeatability was evaluated from 2 tests on each cart over 5 days. Ethanol burning test results are reported in Table 2. During the combustion of ethanol (5mL), the total O_2 and CO_2 measured by Max II -Cart A, was within 99-101% of the theoretical estimates and was not different from the total O_2 and CO_2 measured by the reference standard, Deltatrac II. The total O_2 and CO_2 measured by Max II – Cart B, deviated more than 5% from theoretical values and was significantly different from the Deltatrac II (p<0.05).

Accuracy of the Max II metabolic cart in comparison to Deltatrac II. Overall, strong and significant positive correlations were found between each Max II cart and the reference cart, Deltatrac II for VO_2, VCO_2, RQ and RMR (Table 3). Neither slope nor intercept of the regression between the Max II carts (A and B) and Deltatrac II for VO_2, VCO_2, RQ and RMR differed significantly from 1 and 0, respectively (Table 3). Comparisons between Max II carts and Deltatrac II by mixed-models indicated that Max II -Cart A did not differ from Deltatrac II with regard to VO_2, VCO_2, and RMR (p=NS), whereas RQ was slightly but significantly lower by 0.02±0.03 units (p<0.01). On the other hand, Max II -Cart B exhibited a significantly lower VO_2 (-8±12 ml/min, p<0.05) and higher RQ (0.02±0.02 units, p=0.02) but neither VCO_2 (p=0.67) nor RMR (p=0.075) differed from the values obtained with the Deltatrac II.

Reliability the Max II metabolic cart in comparison to Deltatrac II. For repeated measurements with the Deltatrac II, the within-subject coefficient of variation (CV) was 3.6% for VO_2, 4.5% for VCO_2, 3.3% for RQ and 3.7% for RMR. As shown in Table 3, the within-subject coefficient of variations for Max II -Cart A were also less than 5% for each parameter and thus acceptable and comparable to Deltatrac II. The within-subject coefficient of variations for Max II -Cart B was significantly higher for VO_2, VCO_2, and RMR (p<0.05, Table 3). The intra-class correlation coefficients (ICC) were ≥ 0.9 for Max II Carts A and B suggesting a good agreement between the instruments across the 2 study visits.

Bland-Altman analysis for VO_2, VCO_2, RMR and RQ between the reference cart (Deltatrac II) and Max II -Cart A (Figure 2) showed no systematic bias of the Max II Cart A for measurement of any of the parameter, suggesting good agreement between the instruments. On the other hand, a systematic bias was found for VCO_2 on cart B (r=0.56, p=0.02), as well as a significant mean difference in VCO_2 and RQ, therefore suggesting poor agreement between Max II -Cart B and the reference cart (Deltatrac II).

DISCUSSION

We conducted a controlled randomized, crossover and counterbalanced study in a group of 22 adults to test the validity of the Max II metabolic cart for the measurement of oxygen consumption (VO_2), carbon dioxide production (VCO_2), respiratory quotient (RQ) and resting metabolic rate (RMR) during a 30 minute resting metabolic rate test. We also compared the accuracy of each with a standard combustion of 5 mL of ethanol. Under controlled laboratory conditions we determined: 1) Max –Cart A but not Max Cart B could accurately measure of oxygen consumption (VO_2), carbon dioxide production (VCO_2), respiratory quotient (RQ) and resting metabolic rate (RMR), 2) as expected based on this finding, Max II –Cart A provided valid assessments of resting metabolic rate in comparison to Deltatrac II the reference cart, 3) Max II –Cart B did not provide accurate assessments of resting metabolic rate in humans, and 4) measurement of resting metabolic rate with Max II metabolic carts are reproducible. The study demonstrated the clear importance of conducting quality control testing using standard tests, such as the combustion of ethanol, and to not only rely on the use of daily calibrations to assume analyzer accuracy. The Max II metabolic cart may provide a valid alternative to the Deltatrac II for resting metabolic rate testing in humans.

An opening in the bedside calorimeter market was created as a result of the discontinuation of the Deltatrac II metabolic cart. In an attempt to fill this void, over the last decade several companies have adapted carts developed for exercise testing to perform as bedside calorimeters to allow for measures of metabolic rate at rest. This adaptation for the most part has proved problematic. In general, the analyzers used to measure the concentrations of oxygen and carbon dioxide in inspired and expired air are accurate regardless of their assigned purpose being for rest or exercise. As long as appropriate calibration gases are used for each purpose and reflect the physiological state being measured, the linearization of the analyzer can be tested. The primary shortcoming for adapting carts designed for exercise for assessments of energy metabolism at rest, is in the air flow programming. The robust feature of the Deltatrac II is the set flow rate. Three different flow rates can be chosen; 20L for pediatric subjects, 40L for adult subjects, 60L for adult obese subjects. The flow is not adjusted throughout the measurement.

While the Max II cart does not fall into the category of adapted fitness equipment, it does have variable flow rate in common with exercise carts which is in direct contrast to the Deltatrac II. For the Max II, flow rate is calibrated prior to each assessment with a calibration syringe and the software calls for adjustments to the flow rate throughout the test to maintain data within the physiological range of measurement. The manufacturer instructions call for $FeCO_2$ to be maintained between 0.6 - 0.95% when assessing metabolic rate at rest. When establishing a standard operating procedure for the device, we found that subtle adjustments to the flow rate throughout a test can result in major alterations in VCO_2, even while maintaining the $FeCO_2$ within the desired range. Therefore, we recommend that investigators record the changes in flow rate that occur during a given test and ensure that flow rates are replicated in repeat tests of the same subject. Within the Max II software, flow rate is recorded every minute and the mean can be taken for reference for repeat tests of the same subject.

When considering Max II –Cart A, with confirmed accuracy for measurement of $\%O_2$ and $\%CO_2$ against the ethanol combustion test, it provided comparable measurements of VO_2, VCO_2, RQ and RMR within the same subject and its performance in comparison to the Deltatrac II was furthermore consistent over time. It is problematic that both of the Max II Carts we tested in this study did not function to the same level of accuracy. Despite this frustration, these data emphasize the clear importance of quality control testing within laboratories and clinical settings using a robust test such as that of ethanol combustion. If we had relied on the assessment of daily calibrations to verify cart performance, we would have been blinded to the clear issues with the analyzers and without a direct comparison with the 'gold standard' would have accepted the data derived by the instrument as accurate, valid and reliable. We should point out that as a result of the consistently poor accuracy of Max- Cart B, the cart was returned to the manufacturer at which time a relinearization of the analyzers was performed. While papers have been published in an attempt to standardize controlled testing for metabolic rate assessments [11], the importance for including routine quality control testing is seldom discussed. As good practice, laboratories should assess the accuracy of their metabolic carts on a monthly basis and the combustion of ethanol is an acceptable methodology [14].

Several other carts on the market that have been compared to the Deltatrac include the QUARK RMR (Cosmed, Rome, Italy) [15], MedGem (Microlife USA, Golden, CO) [16], Metavine (Vine, Tokyo, Japan) [17], TrueOne 2400 (Parvo Medics, Sandy, UT), MedGraphics CPX Ultima (Medical Graphics Corp, St Paul, MN), Vmax Encore 29 System (VIASYS Healthcare Inc, Yorba Linda, CA), and Korr ReeVue (Korr Medical Technologies, Salt Lake City, UT) [10, 16]. We are not suggesting that the Max II necessarily supersedes these other carts on the market, and obviously this kind of statement cannot be made unless a head-to-head comparison between all the instruments is made in the same individuals.

In summary, measurements of VO_2, VCO_2, RQ and RMR derived from the Max II metabolic cart are valid in adults compared to the preferred cart, the Deltatrac II. Laboratories should adopt a standard operating procedures for any cart including careful notation of flow rates during test procedures that can be replicated for repeat tests within subjects, if necessary. In addition, laboratories should adopt quality control testing that can assess the performance or accuracy of the O_2 and CO_2 analyzers such as ethanol combustion tests. This validation study highlights that not all metabolic carts, even from the same manufacturer, perform equally. Importantly, this study highlights that ignoring quality control testing can produce invalid measurements of RMR and substrate oxidation that otherwise would go undetected.

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Author contributions

LMR, principle investigator, designed the study, supervised data collection and wrote the paper; EAF conducted the study analyzed data and contributed to writing the paper; VL analyzed the data and contributed to writing the paper; JAS performed quality control tests and supervised data collection, ER co-principle investigator involved in data interpretation, writing of the manuscript.

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Figure Legends

Figure 1. Study design and testing schematic. M_A and M_B are 30-min repeated tests on 2 Max II metabolic carts (A and B) and D is a 30-min measure on a Deltatrac cart

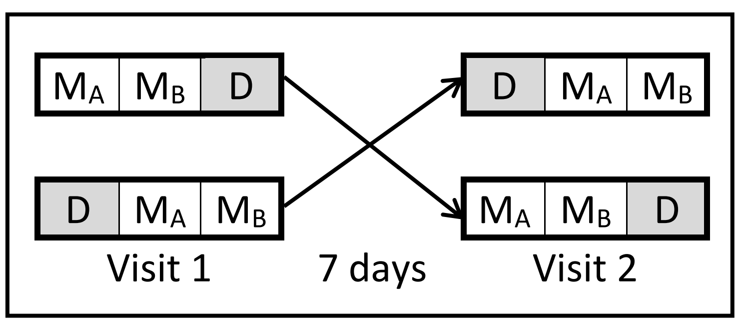

Figure 2. Bland-Altman plots for measurements of VO_2 (A), VCO_2 (B), RQ (C) and RMR (D) obtained with the Max II metabolic cart A in comparison to Deltatrac II.

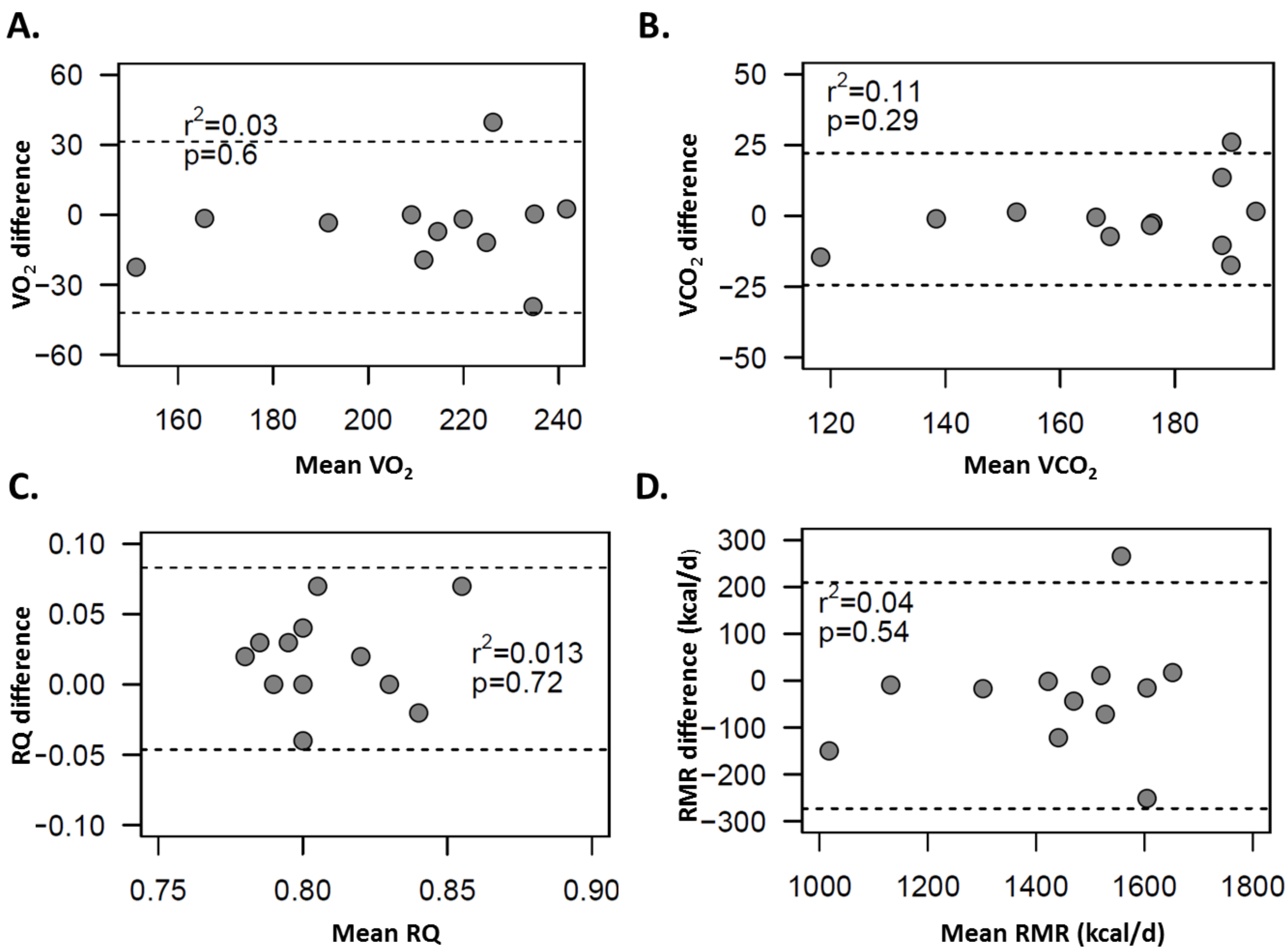

Table 1. Subject characteristics

	Males (n=10)		Females (n=12)	
	Mean ± SD	**Range**	**Mean ± SD**	**Range**
Race (C/AA/O)	6/4/0		5/6/1	
Age (y)	32.9 ± 13.0	21 - 59	37.3 ± 12.7	20 - 58
BMI (kg/m^2)	27.1 ± 5.0	22.4 - 36.9	27.2 ± 6.9	19.2 – 42.0
Weight (kg)	85.8 ± 17.3	66 – 117	71.5 ± 16.7	52 - 110
Height (cm)	177.6 ± 3.1	172 – 182	162.5 ± 6.0	154 - 174
Fat mass (kg)	20.7 ± 8.4	9 - 34	29.5 ± 12.6	16 – 58
Fat free mass (kg)	65.3 ± 10.7	57 - 85	42.4 ± 5.1	35 - 52
RMR (kcal/24hr)	1725 ± 218	1501 - 2153	1304 ± 174	993 - 1562

Race: Caucasian/African American/Other; RMR reported from Deltatrac II^{TM}

Table 2. Accuracy of the oxygen and carbon dioxide analyzers to combust 5mL of ethanol: A comparison between Max II and Deltatrac II (reference cart).

	Mean ± SD	CV
Deltatrac II		
Oxygen Recovery	101.3% ± 1.1%	1.07%
Carbon Dioxide Recovery	101.4% ± 1.9%	1.85%
Respiratory quotient	0.66 ± 0.01	1.48%
Max II - Cart A		
Oxygen Recovery	100.9% ± 3.7%	3.63%
Carbon Dioxide Recovery	99.7% ± 2.9%	2.90%
Respiratory quotient	0.66 ± 0.01	1.67%
Max II – Cart B		
Oxygen Recovery	95.7% ± 1.4%*	1.43%
Carbon Dioxide Recovery	95.2% ± 2.2%*	2.31%
Respiratory quotient	0.66 ± 0.01	1.58%

Theoretical values are 100±0.03% for O_2% and CO_2% and 0.67±0.03 for RQ. CV: coefficient of variation for measures across 10 tests. *denotes significant difference from reference cart and significant difference from theoretical values for ethanol combustion.

Table 3. Comparison of the correlation between the Max II metabolic carts (Carts A and Cart B) and Deltatrac II, the reference cart.

		Correlation		Slope			Intercept			Versus Deltatrac II	
	Max II	r^2	p-value	Slope	Std Error	p-value	Intercept	Std Error	p-value	CV (%)	p-value
VO_2	Cart A	0.87	<0.001	1.01	0.100	0.92	2.7	21.9	0.90	4.2	0.48
	Cart B	0.88	<0.001	1.02	0.097	0.85	-10.8	21.6	0.63	5.0	**0.03**
VCO_2	Cart A	0.9	<0.001	1.10	0.095	0.31	-18.9	16.9	0.28	4.9	0.93
	Cart B	0.93	<0.001	1.15	0.081	0.08	-10.8	14.5	0.07	6.7	**0.03**
RQ	Cart A	0.62	0.005	0.62	0.19	0.06	0.28	0.15	0.08	3.9	0.96
	Cart B	0.43	0.004	0.78	0.23	0.36	0.20	0.19	0.31	4.0	0.13
RMR	Cart A	0.88	<0.001	1.03	0.098	0.78	-16.0	146.7	0.92	4.3	0.48
	Cart B	0.89	<0.001	1.04	0.093	0.64	-108.1	141.4	0.46	6.1	**0.02**